INFLUENCE OF TOOTH AGE AND APPLICATION TIME ON THE MICROTENSILE BOND STRENGTHS OF DIFFERENT ADHESIVE SYSTEMS TO DENTIN

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ABSTRACT

GREICE OLIVEIRA: Influence of tooth age and application time on the microtensile bond strengths of different adhesive systems to dentin.

(Under the direction of André V. Ritter)

This study evaluated the effect of tooth age and application time on the microtensile bond strengths (µTBS) of different adhesives to dentin. One-hundred twenty intact human teeth were mechanically ground to expose mid-coronal dentin and randomized to three groups (n=40) according to subject's age in years: 15-25, 35-45, and ≥55. Within each group, specimens were further randomized to 8 experimental subgroups according to adhesive (etch-and-rinse 3- and 2-step; self-etch 2- and 1-step) and application time (instructions vs. extended). Composite resin was applied to the treated surfaces and after storage for 24 hours all specimens were processed for µTBS testing. Data were analyzed by factorial ANOVA and post-hoc tests (p=0.05). µTBS values ranged from 10.9 MPa (2-step self-etch, extended application time, age group 15-25) to 50.7 MPa (1-step self-etch, extended application time, age group ≥55). Tooth age and application time had no significant effect on µTBS of the adhesives tested.

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INTRODUCTION

Adhesive dentistry has become increasingly popular over the last two decades. Adhesive dentistry not only allows clinicians to use esthetic materials such as composites and porcelains, but also permit a more conservative tooth preparation than with non-adhesive restorations, since adhesive systems reduce the need for conventional mechanical retention forms.

Enamel bonding is successful and very predictable. Bonding to dentin is more challenging because of its more complex structure and composition. Dentin hybridization - which is accomplished by the penetration of resin monomers into the dentin matrix – is considered the main dentin bonding mechanism.[1]

Adhesive systems can be classified according to their bonding mechanism. They can either completely remove the smear layer or incorporate it into the hybrid layer. This is achieved by etch-and-rinse and self-etch systems, respectively. Etch-and-rinse adhesive systems are still considered the gold standard and can achieve immediate bond strengths similar to that of enamel. Self-etch adhesives have become popular between clinicians for two main reasons: the duration of the procedure is reduced and post operative sensitivity is less frequent.

Before adhesive systems were launched on the market, *in vitro* studies were typically done to measure and evaluate the bond strengths of various systems. Most dentin bonding studies use a standard protocol regardless of the type of dentin substrate. The effects of physiological and pathological changes of the dentin have not been extensively studied.

LITERATURE REVIEW

Dentin

Dentin is a mineralized tissue formed by odontoblast cells, and comprises a significant portion of the tooth structure.[2] Human dentin contains approximately 50% inorganic material, 30% organic component, which consists of mainly fibrillar mainly Type I collagen organic material, and 20% water. [3]

Dentin contains tubules, which correspond to the path taken by odontoblast process extending from the pulp towards the dentino-enamel junction (DEJ) and cementum.[4] These tubules have irregular walls with microchannels that are connected to neighboring tubules. [5] The inner wall of dentin tubules is a collagen-poor, hypermineralized cuff known as peritubular or intratubular dentin. [6-7] Tubule course, number and dimension vary according to their location. [8] They are tapered, with their wider portion is near the pulp. [8] The tubule area near the pulp corresponds to approximately 22%, while at the DEJ, corresponding to 1%. The diameter of the tubules is about 2.5 µm near the pulp, and 0.8 µm at the DEJ. Also, the intertubular area differs from predentin to the DEJ. It corresponds to 12% and 96% respectively. Thus, peritubular dentin decreases from about 60% to 2.9% at the DEJ. [5]

In addition, tubules permit fluid movement. An outward flow of dentinal fluid occurs within the tubules because of a small but positive pulpal pressure, which is approximately 10 mm Hg or 15 cm H_2O. [9] The dentin sensory mechanism is related to the hydrodynamics of dentinal and pulpal fluids. [10] When exposed to external stimuli, fluid moves inside the tubules, activating pulpal nerves producing sensitivity and pain. [11] A study carried out by Brännström et al. showed that clinical procedures such as the use of rotary instruments on dentin results in expansion and outward movement of fluid within dentin tubules. [12]

Hydraulic conductance varies at different areas of the tooth. In areas with reduced amount of dentin and wider tubules, dentin permeability is increased. For example, deep dentin is more permeable than superficial dentin. Also, occlusal dentin is less permeable at the DEJ than close to the pulp horns. [13-14] (Tables 1 and 2)

Dentin age

Physiological and pathological changes are a normal consequence of dentin aging. [4] As teeth age, calcification of the tissue continue, promoting narrowing of the dentinal tubules. Formation of reparative dentin also occurs near the pulp. Reparative dentin has less tubules and a more irregular structure. This calcification can occur not only as a normal part of aging (physiological sclerosis), but also as a response to external stimuli such as attrition, caries, erosion or abrasion. [15] As the composition of the dentin might affect the performance of adhesives, some might bond better to a hypermineralized tissue and others to a more organic substrate. [16] Differences in the structure and physiology of the dentin present on prepared surfaces play a fundamental role in the quality of the bond that can be achieved by the different types of bonding systems. [17-19]

Given that hybridization of the intertubular dentin with resins is thought to be the main bonding mechanism, it would be expected that bonding to deep dentin would be less effective than bonding to the dentin near the DEJ, because the area occupied by tubules near the pulp is greater when compared with superficial dentin. [20] For a similar reason, considering the physiology of dentin, large tubules present in young teeth would reduce the bonding outcome of adhesive systems. Both the less mineralized tissue and the opened of the tubules (contributing for contamination of the surface) could contribute to it. [21]

Dentin adhesion

It is generally accepted that adhesive restorative and preventive dentistry was introduced when Buonocore, in 1955, suggested that acids could modify the enamel surface, allowing it to become more receptive to adhesion. [22]

Bonding to enamel is a relatively simple process. In contrast, adhesion to dentin is more difficult and less predictable than that achieved by enamel. Because of the complex dentin structure and also its variable composition, dentin adhesion is more challenging. While enamel is mainly composed of carbonate-rich hydroxyapatite (about 92%), dentin contains a greater percentage of water and organic material. [23]

Dentin bonding or adhesion refers to the micromechanical coupling or union of restorative materials to dentin, particularly dental composites, via an intermediary adhesive resin layer. [24]

Etching of the dentin was initially proposed in 1956 by Buonocore and colleagues [25]. However, the technique did not become popular until improved resin monomers were introduced and phosphoric acid etching was recommended by Fusayama in the late 1970's. [26] Over the years, dentin bonding has been improved with the development of modern dentin bonding adhesives. Formation of a smear layer produced by instrumented dentin is considered one of the main concerns in bond strengths. It leads to a difficult interaction between the adhesive system and the underlying dentin. [19] The smear layer, when not removed, reduces dentin permeability by occluding the tubules. The smear layer prevents fluid movement within the tubules by 86%. [27] The smear layer is mainly composed of hydroxyapatite and altered denatured collagen. [28] Bonding between smear layer and dentin substrate is only about 5 MPa. [29]

Dentin is permeable within the tubules (intratubular permeability) and also at the intertubular surface of these tubules (intertubular permeability) when demineralized with acid. Both types of dentin permeability are crucial to establish an adequate bond. Because dentin near the pulp contains a greater amount of tubules, intratubular permeability will be critical for bond strength in this area. Likewise, intertubular permeability plays a major role in adhesion at the superficial dentin. [30]

The hybrid layer was first described in 1982 by Nakabayashi. When an adhesive is applied over partially demineralized dentin, the mineral component is replaced by resin monomers. These monomers interconnect with collagen fibers resulting in a hybrid structure of biological and artificial polymers. The formation of the hybrid layer or resin-dentin interdiffusion zone is considered the major mechanism of bonding. [1, 31] In summary, micromechanical interlocking occurs after dentin demineralization, resin monomer infiltration, and polymerization. Thus, effective smear layer removal in addition with adequate demineralization of dentin, good wetting, dispersion, penetration and polymerization of the resin components are critical factors to achieve optimal adhesion.[32]

Adhesive systems

Many adhesive systems have been developed during the last few decades.[33] Resin-dentin adhesive systems are classified according to their strategy of interaction with the smear layer: etch-and-rinse technique (etch-and-rinse) or self-etch technique. [34-35] Also, dentin adhesives are classified according to the number of procedures required: three-step, two-step, or one-step (all-in-one).

The main characteristic of etch-and-rinse adhesives is the complete removal of the smear layer. Acid-etching removes the smear layer, expose the intertubular and intratubular dentin, and increases dentin permeability, allowing resin infiltration into the partially demineralized dentin surface and sub-surface. [23] [34] Etching (typically with 30-40% phosphoric acid), also promotes demineralization of the underlying dentin to improve the contact between adhesive monomers and collagen fibers. In three-step etch-and-rinse systems, altering the acid, a primer is used to facilitate the wetting of the adhesive onto the dentin surface, and then a bonding resin is applied. Etch-and-rinse two-step, which is a simplified system, combines both primer and resin into a single solution. [36] Primers contain monomers dissolved in solvents (water, acetone or alcohol). The solvent allows penetration of resin monomers into the collagen fibers. The bonding agent usually contains some type of monomer that infiltrates the tubules, creating an intimate contact with the conditioned tissue substrate, polymerizes, eventually promoting bonding between dentin and restorative material. [24] Three-step etch-and-rinse adhesives are considered the gold standard in dentin bonding.

Wilder *et al.* conducted a 12-year clinical trial to evaluate the performance of a dual-cured three-step dentin adhesive (OptiBond Dual Cure, Kerr; no longer on the market). Fifty-three patients were randomly assigned to two groups according to the etching method (enamel only, or enamel and dentin). One hundred restorations were placed (50 in each group). Evaluation was performed at baseline, and after 1 year and 12 years after placement using a modified USPHS. After 12 years, 46 restorations were evaluated. The retention rates were 93 percent for restorations that were etched only in enamel and 84 percent for those etched in enamel and dentin. The overall retention rate was 89 percent after 12 years of service. [38]

Ritter *et al.* recently published results of an eight-year clinical trial comparing the performance of two etch-and-rinse two-step adhesives (OptiBond Solo, SDS Kerr; and Prime & Bond 2.1, Dentsply Caulk). Ninety-nine restorations were placed in 33 patients. Most of the patients received both types of restorations. Restorations were evaluated following a modified U.S. Public Health Service (USPHS) criteria at baseline, six months, 18 months, 36 months and eight years after restoration placement. After eight years, 56 restorations were assessed. The retention rate was 69 percent for OptiBond Solo and 59 percent for Prime & Bond 2.1. The performance of both adhesives was considered good after eight years of service. [37]

Self-etch adhesive systems have become increasingly popular in clinical dentistry. They reduce the clinical application time, and are less technique-sensitive than etch-and-rinse adhesives. [39] The acid-etching step is eliminated, so acid and primer are incorporated in a single solution and a final bonding agent is applied later. A hybrid layer including both the smear layer and underlying dentin is formed. All components - acid, primer, and resin adhesive - can be available in one solution (self-etch one-step or all-in-one). [24, 40] Self-etch adhesives can be subdivided into two groups: strong (pH 1 or below) or mild (pH around 2). Strong self-etch adhesive have a similar bonding approach of etch-and-rinse adhesives, whereas mild adhesives acts removing the smear layer partially. [39, 41-43]

Self-etch adhesives are anecdotally associated with less post-operative sensitivity when compared with etch-and-rinse adhesives. However, Perdigao *et al.* reported a clinical study comparing the effect of postoperative sensitivity of etch-and-rinse and self-etch adhesives. Thirty-four restorations were done with etch-and-rinse adhesive and 30 with self-etch adhesive. Each patient received at least two restorations and the type of adhesive to be used was randomly assigned to each tooth. A hybrid composite was the restorative material used. Restorations were performed by two operators and were evaluated at 2 weeks, 8 weeks and 6 months post-operatively. The authors found no significant differences in postoperative sensitivity between etch-and-rinse and self-etch adhesives. [35]

Application time of products can influence the bond strength of dentin adhesives. It is evident that varying the treatment of dentin surface will alter the mineral content of the substrate. [2] Increasing acid etching can promote a greater dentin demineralization. Nevertheless, over-etching the dentin can create a deeper zone making effect penetration of priming agents more difficult. Indeed, the formation of a more uniform hybrid layer is more important than the depth of demineralization to achieve a stable dentin bonding. [30, 44]

Microtensile bond strength test

Effectiveness of dentin adhesive systems has been reported through *in vitro* and clinical studies. Bond strength tests have high variability.[2] Some studies have a poorly described methodology and, due to lack of standardization, comparison between results may not be possible. [45] Long-term clinical trials are the ultimate method to evaluate effectiveness of dentin adhesives.[34] However, laboratory tests are essential as screening tests to evaluate new adhesive products and test conditions.[33] Bond strengths can be measured using different types of tests such as macro- or micro-test set-ups, which are related to the bond area to be tested. Macro-bond strengths can be tested with different methods – shear, tensile, or push-out.[46] Shear bond strength testing is the most frequently used test.[45] However, in our *in vitro* study we opted to use a microtensile bond strength test. The microtensile bond strength method of bond strength testing was developed in

1994 by Sano and has several advantages over macrotensile tests: better stress distribution at the bonding area, improved comparison of data from peripheric and central dentin, and the ability for collection of multiple microspecimens from each tooth.[47-48] Indeed, during analysis of the gathered data, it is necessary to account for correlations between microspecimens and not count them as independent rods with the aim to avoid overstating the final result.[49]

SPECIFIC AIMS

1. To evaluate the effect of dentin age on the microtensile bond strengths of different adhesive systems to dentin.
2. To evaluate whether different application times influence the microtensile bond strengths of different adhesive systems to young vs mature dentin.

NULL HYPHOTHESES

1. The age of the tooth has no effect on the microtensile bond strengths of different adhesive systems to dentin.

2. Different application times have no effect on the microtensile bond strengths of different adhesive systems to young versus mature dentin.

MATERIALS AND METHODS

One hundred and twenty extracted intact and caries-free human molars and premolars were collected from five different dental clinics (Durham, NC; Dunn, NC; Sanford, NC; Rocky Mount, NC; and Tempe, TX); a Missions of Mercy clinic in Tarboro, NC; and from the Veteran Affairs Medical Center in Durham, NC. Many of the specimens were third molars, premolars extracted for orthodontic treatment, or teeth extracted due to periodontal disease.

The experimental design is presented in a flowchart (Figure 1).

The specimens were stored in an aqueous solution of 0.2% chloramines trihydrate, which acts as an antibacterial agent, for no longer than 6 months prior to testing. Upon extraction, each tooth was individually stored in glass containers and labeled with the patient's age and date of extraction (Figure 2). Specimens were assigned to three groups of 40 specimens each according to the patient's age (used as a surrogate measure for the tooth age): 15 to 25 years, 35 to 45 years, and 55 years or older. These age groups were selected in order to provide (1) representation of young (15-25), moderate (35-45), and mature (55 years or older) dentin, and (2) enough separation between the age groups to avoid bias due to overlapping ages.

The pulp chamber was accessed through the root ends and the pulp tissue was removed. Using an Ecomet 3 Grinder/Polisher machine (Buehler, Lake

Bluff, IL.) (Figure 3), the occlusal enamel of each specimen was mechanically ground with water-cooled sandpapers, and mid-coronal dentin was exposed (Figure 4 and 5). The peripherical enamel was completely removed with burs (diamond #849, Brasseler USA, Savannah, GA) (Figure 6). The exposed flat dentin surface was polished using 600-grit silicon carbide paper under running water for 10 seconds to obtain a standardized smear layer.

Within each age group, the specimens were randomly divided into 8 experimental subgroups according to the adhesive system and the application technique used:

- Subgroup 1: Etch-and-rinse, 3-step (ER3-IFU, Adper Scotchbond Multi-Purpose Plus, 3M ESPE, St. Paul, MN); acid-etching time following manufacturer’s instructions. Following application of 37% phosphoric acid gel for 15 seconds, specimens were rinsed with tap water for 15 seconds and blot-dried with an absorbent paper for 5 seconds, leaving a moist surface. Primer was applied and gently air-dried for 5 seconds. If the surface did not appear shiny, an extra layer was applied and dried. One coat of Adper Scotchbond Multi-Purpose adhesive was applied to the dentin and was light-cured for 10 seconds.
- Subgroup 2: Etch-and-rinse, 3-step (ER3-IFUX2, Adper Scotchbond Multi-Purpose Plus, 3M ESPE); double acid-etching time recommended by the manufacturer. Same application protocol as in Subgroup 1, with double etching time (i.e., 30 seconds).

- Subgroup 3: Etch-and-rinse, 2-step (ER2-IFU, Adper Single Bond Plus, 3M ESPE); acid-etching time following manufacturer's instructions. Specimens were etched with 37% phosphoric acid gel for 15 seconds and rinsed with tap water for 10 seconds. Excess water was blottled with absorbent paper for 5 seconds, leaving a glistening surface. Immediately after blotting, 3 consecutive coats of Adper Single Bond adhesive were applied to the etched dentin for 5 seconds with gentle agitation with a fully saturated applicator. After gentle air-thining to evaporate the solvent, the adhesive was light-cured for 10 seconds.
- Subgroup 4: Etch-and-rinse, 2-step (ER2-IFUX2, Adper Single Bond Plus, 3M ESPE); double acid-etching time recommended by the manufacturer. Same application protocol as in Subgroup 3, with double etching time (i.e., 30 seconds).
- Subgroup 5: Self-etch, 2-step (SE2-IFU, Adper Scotchbond SE, 3M ESPE); primer application time following manufacturer's instructions. Specimens were rinsed with tap water, and lightly dried with an absorbent paper for 5 seconds. Liquid A (aqueous primer) of Adper Scotchbond SE was applied so that a red-colored layer was obtained on the dentin surface. Following it, Liquid B (acidic adhesive) was applied, making the red color disappear quickly. This color change indicates that the etching components were activated. Liquid B was scrubbed for 20 seconds with moderate finger pressure. The bonding agent was air-dried thoroughly for 10 seconds to evaporate water. A second layer of Liquid B was applied and gently air-

thinned to adjust the film thickness and consistency. The adhesive was light-cured for 10 seconds.

- Subgroup 6: Self-etch, 2-step (SE2-IFUX2, Adper Scotchbond SE, 3M ESPE); double primer application time recommended by the manufacturer. Same application protocol as in Subgroup 5, except Liquid B was applied for 40 seconds.
- Subgroup 7: Self-etch, 1-step (SE1-IFU, Adper Easy Bond, 3M ESPE); adhesive application time following manufacturer's instructions. Specimens were rinsed with tap water, and lightly dried with an absorbent paper for 5 seconds. Adper Easy Bond adhesive (L-Pop) was applied on the dentin for 20 seconds. The disposable applicator was rewetted with adhesive as needed during the application. Subsequently, the adhesive layer was air-thinned for 5 seconds. The adhesive was light-cured for 10 seconds.
- Subgroup 8: Self-etch, 1-step (SE1-IFUX2, Adper Easy Bond, 3M ESPE), double adhesive application time recommended by the manufacturer. Same application protocol as in Subgroup 7; however the adhesive was applied for 40 seconds.

For all specimens in all groups, Filtek Supreme Plus (3M ESPE); shade A2 Body, was used to form composite build-ups. Two to three 2-mm thick composite increments (Figure 7) were placed, resulting in a 4 to 6 millimeter composite build-up (Figure 8). Following the application of the last composite increment, the material was light-cured for 40 seconds (Figure 9). A light-curing unit (Spectrum 800, Dentsply) was used to light-cure the adhesives and composite. Light intensity was monitored daily (≥ 550 mW/cm^2). The specifications (lot, ref, expiration date) and manufacturer's instructions for use of the adhesives and composite used are detailed in Table 1.

After the build-up was completed, specimens were stored in deionized water for 24 hours at room temperature. Then, using a Isomet 1000 diamond micro-slicing saw (Buehler, Lake Bluff, IL) (Figure 10), specimens were sectioned parallel to their long axis into rods (approximately 1 X 1 mm) (Figure 11); half of each rod consisted of composite resin and the other half of dentin (Figure 12). An average of 16 rods per tooth was obtained. An electronic digital caliper (Fisher Scientific, Pittsburgh, PA) was used to measure the cross-sectional area of the dentin-composite interface in each rod, i.e., the bonded area (Figure 13).

The specimens were carefully positioned in a custom notched jig so the forces applied to each rod would be perpendicular to dentin-composite interface, allowing it to be evenly distributed. A cyanocrylate adhesive (Dental Ventures of America Inc, Corona, CA) was used to fix the rods in the jig (Figure 14). The adhesive was sprayed with Zapit Accelerator Spray (Dental Ventures of America Inc, Corona, CA) to accelerate its setting process. Each rod was loaded at a crosshead speed of 0.5 mm/min using an Instron universal testing machine (Model 4411. Canton, MA.) (Figure 15) with a 500N load cell. To determine of the dentin microtensile bond strength (µTBS). The µTBS was calculated from the force recorded at specimen failure divided by the bonding area (mm^2) and was expressed in megapascal (MPa). The bond failure was evaluated and classified as interfacial, cohesive dentin, cohesive composite, or mixed (combination of interfacial and cohesive dentin or composite). Pre-test failures were counted as 0 MPa bond strengths. Results from rods obtained from the same tooth were averaged, so that the tooth (and not each rod) was used as the unit of analysis, because rods coming from the same tooth are not independent specimens. Normality of the data was analyzed with a histogram function. Pairwise correlations between independent and dependent data were analyzed using Stata 10.1 (StataCorp, College Station, TX). Outcomes data were analyzed statistically by a factorial ANOVA and Tukey post-hoc statistical tests (p=0.05) using Statistica 5.5 (StataSoft Inc, Tulsa, OK).

RESULTS

A total of 120 teeth provided 1998 rods, resulting in a mean of about 16 rods per tooth. The distribution of the µTBS values was found to be normal using a histogram function (Figure 16).

Negative linear relationships were found between µTBS and area (r=-0.0494, p=0.0418), µTBS and width (r=-0.0396, p=0.1023), and µTBS and thickness (r=-0.0402, p=0.974). The negative relationship between µTBS and area indicate that the smaller the rod area, the higher the µTBS values. However, the scatter plot graph (Figure 17) does not show a clear correlation between µTBS and area.

The mean microtensile bond strength values and standard deviations for each experimental condition are presented in Table 2. The adhesives tested were not affected by age, except for TE2-IFU that showed significant higher µTBS values for age group ≥55 (46.5 MPa) compare to the younger age groups. Doubling the application time recommended by the manufacturer did not influence the µTBS in any adhesive system. When comparing adhesive systems within each age group, SE2 had significantly lower µTBS for all age groups regardless of the application time. SE1-IFU and SE1-IFUX2 did not result in µTBS values significantly different than SE2 in the age groups 35-45 and ≥55, respectively. SE1-IFUX2 tested in age group ≥55 presented the highest µTBS value (50.78 MPa), while SE2-IFUX2 tested in age group 15-25 presented the lowest µTBS value (10.91 MPa).

Failure modes (interfacial, cohesive dentin, cohesive composite, or mixed) are reported in Table 3. Pre-test failures were counted as 0 MPa. A table including the number of pre-test failures for each subgroup is presented in Table 4. SE2 adhesive showed a higher prevalence of pre-test failures for all age groups when compared with the other adhesive systems.

DISCUSSION

The results of our study revealed no clear relationship between microtensile bond strength obtained by any of the adhesive systems used and the age of dentin. Only a few studies have evaluated the correlation between microtensile bond strength and dentin age, and our results are in agreement with most of these studies.

Tagami *et al.* used four etch-and-rinse adhesive systems to investigate wether their microtensile bond strength values would vary in young (9-21 years) and old (42-64) dentin. All adhesives tested had similar bond strengths despite the effects of aging.[50]

A study carried out by Burrow *et al*. investigated the influence of dentin age and depth on the bond strength of three adhesive systems. Only one adhesive (Superbond D-liner, Sun Medical Co. Kyoto, Japan) was found to be affected by dentin age. Overall, little variation in bond strength was found between young and old dentin.[51]

Ozer *et al*. tested two self-etch adhesives and compared the bond strengths in three age groups (20-25, 35-40, and 50-55 years). One of the adhesives (ABF Bond, Kuraray, Osaka, Japan) showed increased bond strength for older teeth. Reactmer Bond (Shofu, Kyoto, Japan) was not influenced by tooth age.[52]

In 2008, a study by Brackett *et al.* evaluated two adhesive systems and the effect of dentin age using young and aged teeth (over 60 years). They concluded that dentin age did not influence the bond strength of any adhesive tested.[53]

Our findings did not reveal any significant difference when doubling the application time of the products. When reviewing studies that compared different application times of the adhesives that we tested, we found our results to be in agreement with the available studies.

Pioch *et al*. tested five adhesives using different etching times. Scotchbond Multi-Purpose and Scotchbond 1 (Single Bond in the US) did not have any significant different in bond strength when submitted to 15 or 30 seconds of dentin acid etching.[54]

Studies carried out by Lopes *et. al*. showed significant higher bond strength for 30 seconds of acid etching for Single Bond in non carious cervical lesions, and when comparing normal to sclerotic dentin. Doubling etching time in sclerotic dentin achieved similar bond strength of that obtained by normal dentin. [55-56] Also, other studies that evaluate Single Bond, did not find any relationship between bond Strength and prolonged acid etching time when testing sound dentin.[57-58]

Although many microtensile bond strength studies have been done over the last two decades, due to a lack of standardization in study design, it is difficult to establish comparisons between studies. Furthermore, often the methodology is poorly described. Also, *in vitro* studies present several limitations.

In our study, for example, sound teeth were used. This may not reproduce most of the cases in which a tooth needs to be restored. In fact, in a clinical situation, most frequently the substrate available will be a caries-affected dentin.[59] However, we opted for caries-free dentin to facilitate a standardization of the substrate. Caries can be present in different degrees, areas, and depth. Also, smear layer produced by burs are denser than those produced by silicon carbide papers. These factors can directly affect dentin permeability and consequently affect the performance of the bonding agents.[59]

In addition, studying an even younger age group, such as unerupted-10 years, could also be helpful to evaluate whether a much younger dentin substrate would result in substantially different outcomes, because physiological sclerosis and modifications on the structure due to external stimulus are much less likely in that age group.

Although our purpose was to test the adhesive systems using the mid-coronal dentin substrate, standardizing it was extremely difficult. Because tooth anatomy varies widely from tooth to tooth, in many cases the occlusal pit-and-fissure system would be deeper than the average. As a result, after removing all enamel, a deeper dentin would be used. As a suggestion for further research, in the same way that we removed the peripheral enamel with burs, we could also remove the remaining occlusal enamel after the dentin is ground at the desired level (superficial, mid-coronal, or deep dentin). In this case, those rods affected by the bur would be eliminated.

As dentin has a complex structure and different characteristics and variation within a tooth, it is important to take the location of the rods into consideration. In a molar, rods located close to the pulp may result in different bond strength of rods where the intertubular dentin area is increased. Central and peripheric rods were identified for each of the tooth and the results will be addressed and discussed in a future paper.

Moreover, other factors need to be taken in consideration. *In vitro* studies do not take into account the 3-dimensional nature of cavity preparations, leading to an underestimation of the effects of polymerization shrinkage. Internal pulpal pressure, dentinal fluid and tooth dynamics such as flexural phenomena cannot be reproduced. It is known that hydraulic conductance plays a role in adhesion. Mineral content and the diameter of the tubules vary from young to old teeth. Unfortunately we were not able to reproduce or to mimic this condition, eliminating this factor that could be important to determine the difference in bond strength between age groups.

It was clearly observed in this study, during adhesive procedures, an increased dentin permeability in those teeth from young patients. It could be visually noticed especially at those specimens treated with Adper Scotchbond Multi-Purpose Plus. During application of primer, for most of the specimens in the age group 15-25 years, a second or third layer of primer was applied until a shiny surface was obtained. On the other hand, specimens from age group 35-45 years and ≥55 years required 2 or 3 layers. This could be attributed to wider dentin tubules and less mineralized dentin in young dentin, but it was probably exacerbated by the fact that the pulpal tissue was removed prior to the test. The lack of internal pulpal pressure combined with a less demineralized dentin could contribute to a deeper demineralization and a non uniform hybrid layer. Following this rationale, it is possible that specimens from subgroup ER2-IFU could obtain an uniform hybrid layer in a more mineralized tissue. An increased intertubular area may have contributed to a significant higher µTBS value for older teeth. This could be an explanation for lower bond strength in the young age group (15-25 years and 35-25 years) when ER2-IFU was tested.

A SEM study could be done with the adhesives tested to show morphological changes between different dentin age and also compare the formation of hybrid layer with etch-and-rinse and self-etch adhesive systems.

Unfortunately, due to the difficulty in gathering enough specimens and time constraints, we were limited to evaluate the microtensile bond strength of the materials tested only 24 hours after restorations was placed. However, it is known that storage time can influence bond strength values. Over extended periods in water storage, adhesives exhibit evidence of mechanical and morphological degradation which leads to an decrease in bond strength.[60] Prolonged storage time, when possible, should always be considered, since it better mimics the *in vivo* condition.

CONCLUSIONS

Within the limitations of our study, we concluded that:

1. Tooth age did not significantly affect the microtensile bond strengths of the tested adhesive systems to dentin, with the exception of adhesive ER2-IFU, which had significantly higher microtensile values for the age group ≥55 when compared to the other age groups;
2. An extended application time did not significant affect the dentin microtensile bond strengths of the tested adhesive systems.

The study therefore failed to reject the null hypothesis, i.e., tooth age and adhesive application time have no effect on the microtensile bond strength of different adhesive systems to dentin.

TABLES

Table 1. Tubule area and diameter near the pulp and at the DEJ.

	Near pulp	At the DEJ
Tubule area	22%	1%
Tubule diameter	2.5 µm	0.8 µm

Table 2. Intertubular area and tubule diameter at the predentin and at the DEJ.

	Predentin	At the DEJ
Intertubular area	12%	96%
Tubule diameter	60%	2.9%

Table 3. Adhesive systems and composite used: Lot, Ref, Expiration, and manufacturer's direction.

Product	Lot	Ref	Expiration	Manufacturer's Instructions for Use
Adper Scotchbond Multi-Purpose Plus	20081210	7540S	2011-09	Etch: Apply Scotchbond etchant to enamel and dentin 15 seconds. Remove excess water with an air syringe or by blotting. Leave moist. Prime: Apply Scotchbond Multi-Purpose primer to enamel and dentin. Dry gently for 5 seconds (no waiting). Surface will appear shiny. Bond: Apply Adper Scotchbond Multi-Purpose adhesive to enamel and dentin. Light-cure for 10 seconds.
Adper Single Bond Plus	20081203	51101	2010-11	Etch: Apply Scotchbond etchant to enamel and dentin. Wait 15 seconds. Rinse for 10 seconds. Blot excess water using a cotton pellet or mini-sponge. Do not air dry. The surface should appear glistening without pooling of water. Bond: Immediately after blotting, apply 2-3 consecutive coats of adhesive for 15 seconds with gentle agitation using a fully saturated applicator. Gently air thin for 5 seconds to evaporate solvent. Light-cure for 10 seconds.
Adper Scotchbond SE	20090122	42000	2010-11	Bond: Dispense 1 drop of liquid A into one of the mix wells, and 1 drop of liquid B into the second mix well. Wet brush tip with liquid A. Apply to the entire bonding area so that a continuos red colored layer is obtained on the surface. Discard this brush. Wet second brush tip with liquid B, and scrub into the entire wetted surface of the bonding area. The red color will disappear quickly, indicating that the etching components have been activated. Continue scrubbing with moderate finger pressure for 20 seconds to ensure a proper etch. Air dry thoroughly for 10 seconds to evaporate water. Adhesive should remain in place and be shiny in appearance upon completion of this step. Re-coat brush with liquid B, and apply second coat to the entire bonding surface. Lightly air thin adhesive layer to adjust film thickness/consistency. Light-cure for 10 seconds.
Adper Easy Bond	380414	41248	2011-11	Bond: Apply the adhesive with the disposable applicator for 20 seconds to all surfaces of the cavity. Rewet the disposable applicator as needed during application. Avoid contact of the adhesive with mucosal tissue. Subsequently, air thin the liquid for approximately 5 seconds until the film no longer moves, indicating complete vaporization of the solvent. Cure the adhesive for 10 seconds.
Filtek Supreme	20090105	5028A2B	2011-09	Place and light-cure restorative in increments. Avoid intense light in the working field. Shade: Body, enamel and translucent – Increment depth: 2.0mm Cure time: 20 sec. Shade: dentin – Increment depth: 1.5mm – Cure time:40 sec.

Table 4. Microtensile bond strength value (standard deviation) of adhesives by age group and application time with tooth as unit of analysis (including zeros).

		Age groups (years)		
Adhesive	***Application****	***15-25***	***35-45***	***≥ 55***
Etch-and-rinse 3-step	IFU	35.50 (19.71)$^{Aa\blacklozenge}$	44.88 (5.52)$^{Aa\blacklozenge}$	43.75 (3.35)$^{Aa\blacklozenge}$
	IFUX2	30.92 (10.38)$^{Aa\blacklozenge}$	42.36 (10.47)$^{Aa\blacklozenge}$	46.94 (8.13)$^{Aa\blacklozenge}$
Etch-and-rinse 2-step	IFU	28.32 (10.91)$^{Ba\blacklozenge}$	40.15 (14.38)$^{Ba\blacklozenge}$	46.57 (8.46)$^{Aa\blacklozenge}$
	IFUX2	44.23 (12.32)$^{Aa\blacklozenge}$	44.50 (9.39)$^{Aa\blacklozenge}$	50.10 (7.88)$^{Aa\blacklozenge}$
Self-etch 2-step	IFU	11.64 (9.23)$^{Aa\clubsuit}$	18.63 (8.69)$^{Aa\clubsuit}$	23.52 (9.45)$^{Aa\clubsuit}$
	IFUX2	10.91 (3.08)$^{Aa\clubsuit}$	23.43 (7.37)$^{Aa\clubsuit}$	21.34 (9.42)$^{Aa\clubsuit}$
Self-etch 1-step	IFU	38.66 (13.15)$^{Aa\blacklozenge}$	33.42 (12.55)$^{Aa\blacklozenge\clubsuit}$	36.83 (12.47)$^{Aa\blacklozenge\clubsuit}$
	IFUX2	41.46 (9.91)$^{Aa\blacklozenge}$	35.88 (17.39)$^{Aa\blacklozenge\clubsuit}$	50.78 (12.28)$^{Aa\blacklozenge}$

*IFU: instructions for use, IFUX2: instructions for use doubling application time.
Upper case letters compare rows within adhesives (ages in same application) (same letter p>0.05)
Lower case letters compare columns within adhesives (application within ages) (same letter p>0.05)
Superscript symbols compare adhesives within same age and application groups (same symbol p>0.05)

Table 5. Cross-tabulation of mode of failures with age group, adhesive group, and application group.

		Age groups (years)											
		15-25				***35-45***				***≥ 55***			
		Failure modes				***Failure modes***				***Failure modes***			
Adhesive	***Application****	***I***	***cd***	***cc***	***M***	***i***	***cd***	***cc***	***m***	***i***	***cd***	***cc***	***M***
Etch-and-rinse 3-step	IFU	45	14	19	1	59	16	9	0	31	18	10	1
	IFUX2	65	2	6	1	41	11	15	0	27	7	14	0
Etch-and-rinse 2-step	IFU	65	8	8	0	31	21	12	0	20	23	22	0
	IFUX2	42	19	33	1	25	20	22	2	22	11	13	4
Self-etch 2-step	IFU	54	2	0	0	42	7	6	0	62	15	5	2
	IFUX2	79	2	0	0	51	11	5	2	53	7	5	1
Self-etch 1-step	IFU	67	9	12	1	38	15	12	1	46	12	8	0
	IFUX2	71	24	11	0	35	11	12	0	35	17	16	0

*IFU: instructions for use, IFUX2: instructions for use doubling application time.
I: interface, cd: cohesive dentin, cc: cohesive composite, m: mixed.

Table 6. Cross-tabulation of pre-test failures with age group, adhesive group, and application group.

		Age groups (years)								
		15-25			35-45			≥ 55		
Adhesive	***Application****	***Nrods***	***PTF***	***%***	***Nrods***	***PTF***	***%***	***Nrods***	***PTF***	***%***
Etch-and-rinse 3-step	IFU	92	13	14.13	95	11	11.57	62	2	3.22
	IFUX2	82	8	9.75	74	7	9.45	49	1	2.04
Etch-and-rinse 2-step	IFU	97	16	16.49	77	13	16.88	69	4	5.79
	IFUX2	104	9	8.65	72	3	4.16	53	3	5.66
Self-etch 2-step	IFU	83	27	32.53	80	25	31.25	112	28	25.00
	IFUX2	105	24	22.85	94	25	26.59	96	30	31.25
Self-etch 1-step	IFU	94	5	5.31	80	14	17.50	74	8	10.81
	IFUX2	112	6	5.35	70	12	17.14	72	4	5.55

*IFU: instructions for use, IFUX2: instructions for use doubling application time.
PTF: pretest failures.
%: percentage of pretest failures.

FIGURES

Figure 1. Flowchart of study design.

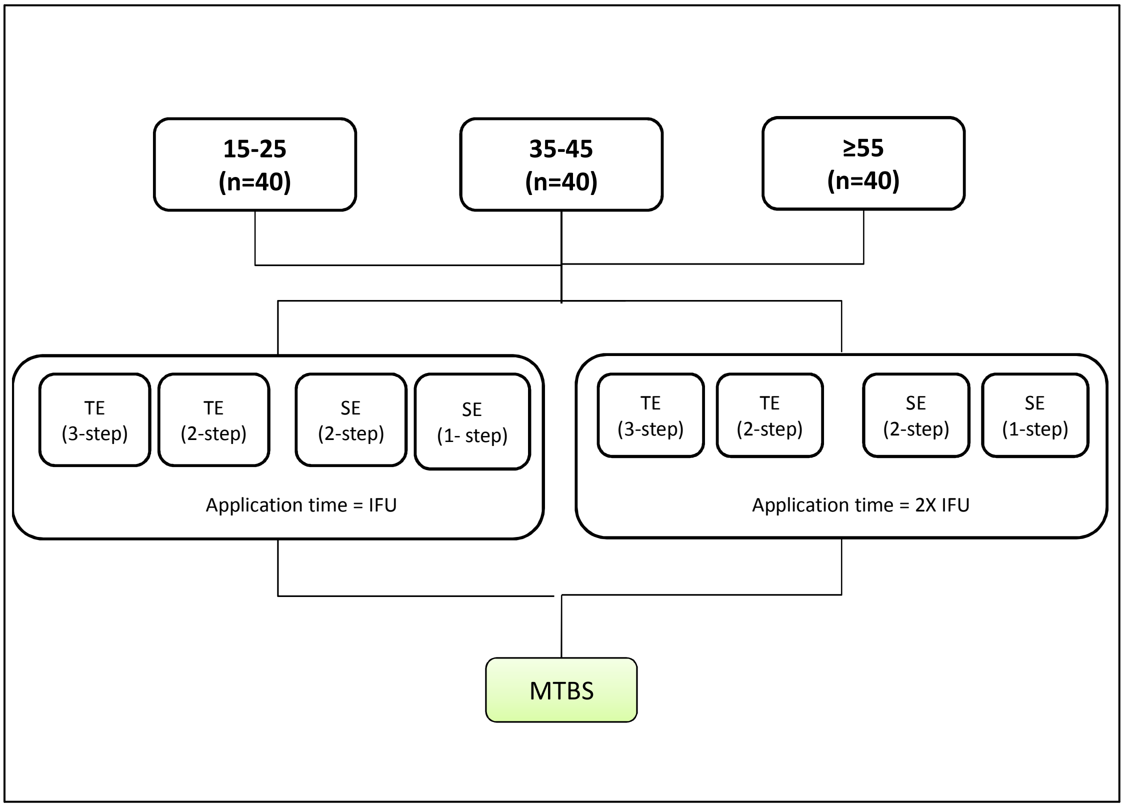


Figure 2. Specimens labeled with patient's age and extraction date.

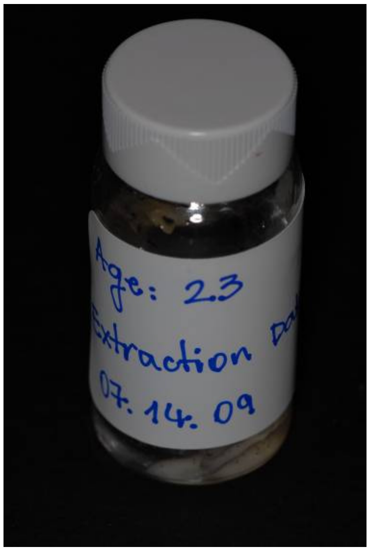


Figure 3. Ecomet 3 Grinder/Polisher machine (Buehler, Lake Bluff, IL.)

Figure 4. Grinding of occlusal enamel of specimen.

Figure 5. Mid-coronal dentin exposed.

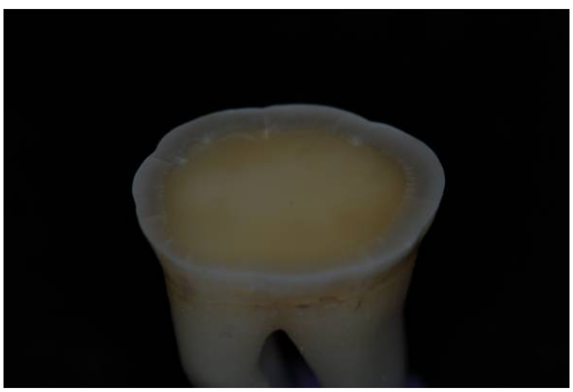


Figure 6. Peripheral enamel was completely removed.

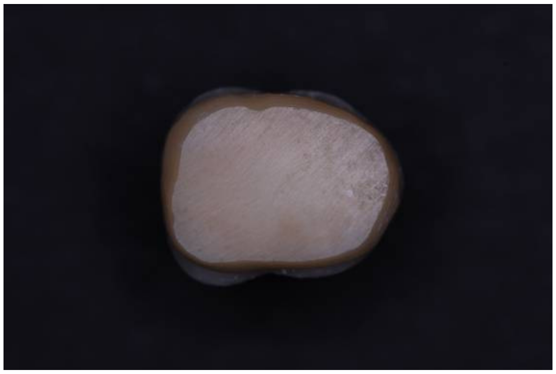


Figure 7. 2 mm composite build-up.

Figure 8. Composite build-up.

Figure 9. Light-curing of composite after last increment placement: 40 seconds.

Figure 10. Isomet 1000 diamond micro-slicing saw (Buehler, Lake Bluff, IL).

Figure 11. Specimens sectioned into rods of approximately 1x1 mm.

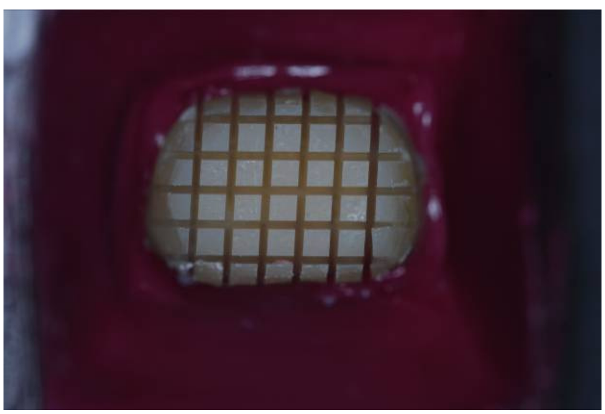


Figure 12. Rods (half composite resin and half dentin)

Figure 13. Measurement of dentin-composite interface with a digital caliper.

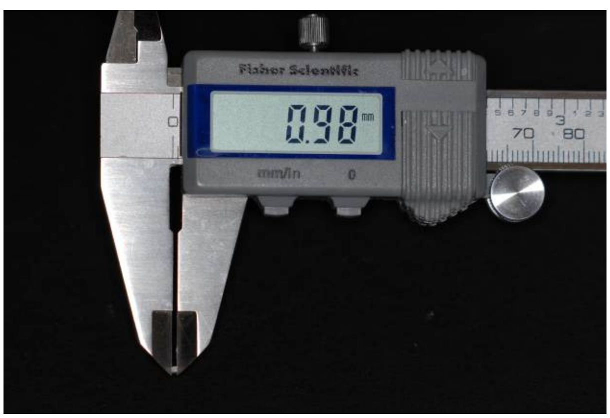


Figure 14. Rod positioned in a custom notched jig.

Figure 15. Instron universal testing machine (Model 4411. Canton, MA).

Figure 16. Histogram function of microtensile bond strength values.

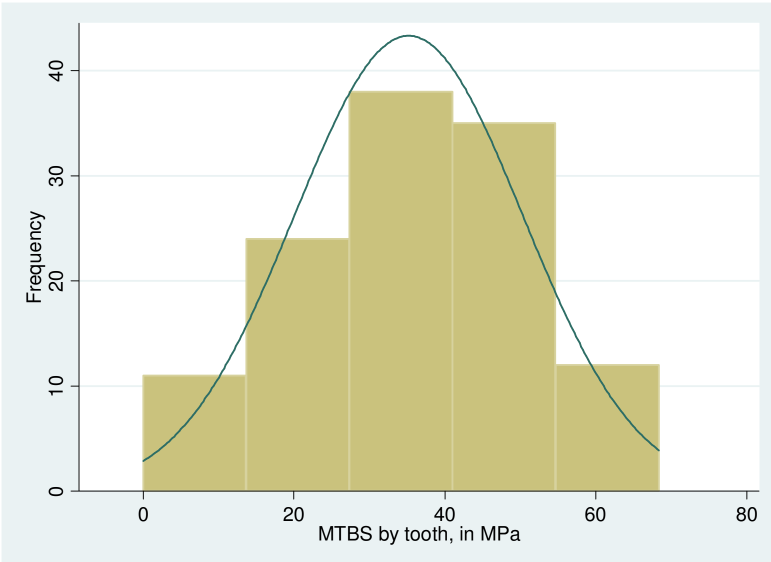
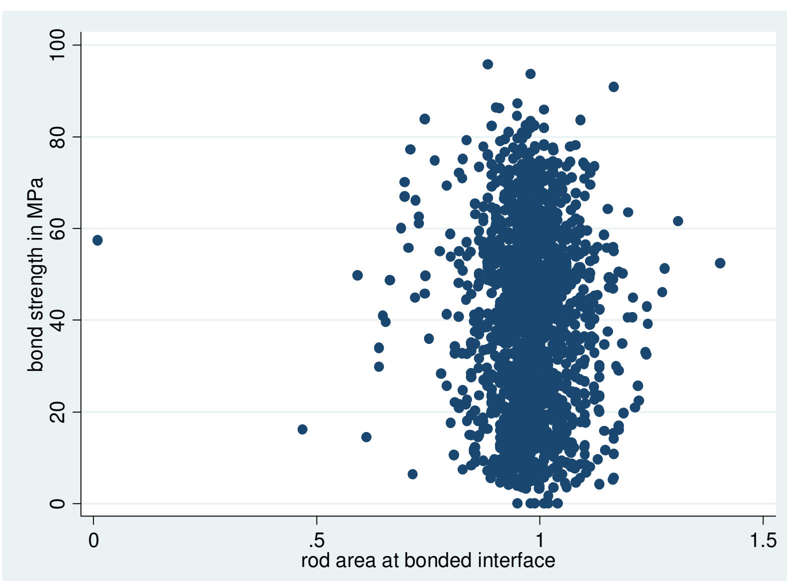


Figure 17. Scatter plot graph presenting relationship between rod area and microtensile bond strength values.



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